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A Longer Life for Yeast with Good Memory

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Cell polarity establishment has been studied in great detail, but much less is known about mechanisms that prevent polarization. Reporting recently in *Cell*, Meitinger et al. (2014) identify an elaborate mechanism in yeast cells that efficiently inhibits Cdc42 activation in cytokinesis remnants. Failure of this "anti-polarization" memory increases replicative aging.

Polarization is a fundamental cellular process that defines a single orientation within a prokaryotic or eukaryotic cell. It is a prerequisite for many developmental and pathogenic processes that include cell migration, epithelial tissue integrity, asymmetric cell division, and tumor development. In the past decade, the budding yeast *Saccharomyces cerevisiae* has been used as a powerful model system to unravel the fundamental mechanisms for the establishment of cell polarity (Freisinger et al., 2013). These studies have identified a complex network of feedback loops that underlie the highly dynamic localization and activation of the conserved polarity regulator Cdc42. While coupled feedback loops are clearly capable of spontaneously breaking symmetry in an idealized cell, it has long been established that yeast cells actually exhibit highly regular patterns of cell division (Casamayor and Snyder, 2002). New bud sites are always chosen adjacent to the previous site of division in haploid cells or in a bipolar fashion in diploid cells. Importantly, division sites become permanently marked upon mother-bud separation and can be identified as birth scars in fresh daughter cells or bud scars in mother cells. The latter are characterized by a chitin-rich rigid cell wall (CW) (Francois et al., 2013) and a set of immobilized integral plasma membrane (PM) proteins, Rax1 and Rax2 (Kang et al., 2004). Importantly, despite bud scars being propagated for many generations (Chen et al., 2000), they are never used as sites of new polarization. In a recent study, it was shown that the Cdc42 GAP Rga1 and the scaffold protein Gps1 act in parallel to inactivate Cdc42 at sites of cell division site after completion of cytokinesis (Meitinger et al., 2013). However, it remained unclear how Gps1 actually influences Cdc42 activity and how Cdc42 is kept inactive on older bud scars (cytokinesis remnants, or CRMs), where neither Rga1 nor Gps1 is present.

In a recent issue of *Cell*, Meitinger et al. (2014) unravel the molecular mechanisms responsible for the establishment of Cdc42 inhibitory zones in CRMs. They show that Gps1 interacts with three additional factors, Nba1, Nis1, and Nap1, and that all four proteins together prevent repolarization of cells at sites of previous cell division. Whereas Gps1 and Nap1 seem to play facilitating roles in cortical recruitment of Nba1 and Nis1, the latter two proteins constitute codependent landmarks that localize to CRMs and directly interfere with Cdc42 activation. Specifically, the inhibitory function of the landmark occurred through interference of Nba1 with activation of the Cdc42 GEF Cdc24 (Figure 1A). Consequently, artificial tethering of Nba1 to septins was sufficient to inhibit repolarization of cells at sites of cytokinesis and at CRMs. The authors propose a model in which Nba1 and Nis1 are recruited to the new cell division site by Gps1 and Nap1 (Figure 1B) and are then transferred to CRMs, where they become permanently anchored to the bud scar landmarks Rax1 and Rax2 (Figure 1C).

This study provides the molecular and structural backbone for the establishment of long-term memory in yeast cells. The identified cortical memory around Nba1 prevents Cdc42 repolarization at sites of previous cell divisions across many generations. At the most recent division site this mechanism acts in parallel to the previously identified inactivation of Cdc42 via its GAP Rga1. In contrast, Nba1 seems to be the only factor inhibiting Cdc42 activation at older CRMs. Importantly, Meitinger et al. (2014) also examined the physiological consequence of disrupting the

memory mechanism and found that cells deficient in establishing refractory Cdc42 activation zones exhibited drastically reduced replicative lifespan. Indeed, cells lacking Nba1 and Rga1 divided on average only 6 times compared to the 20 times observed for control cells. Cell death strongly correlated with nuclear segregation defects, indicating that the reduced lifespan was a consequence of the narrower bud necks in cells that re-bud from the same site.

With its elegant investigation of the molecular mechanisms that generate Cdc42 exclusion zones in yeast cells, the study by Meitinger et al. (2014) provides an exciting example for cellular pattern formation that stands in stark contrast to the concepts developed for cell polarity establishment across recent years. Whereas robust and unique polarization of PM-associated Cdc42 is a highly dynamic process that balances lateral diffusion with rapid recycling (Freisinger et al., 2013), Cdc42 exclusion from bud scars occurs via structural landmarks that remain static over many generations. This is consistent with the need for polarized sites to reset during each cell cycle, or on even shorter timescales during chemotaxis, while the relatively small Cdc42 refractory zones can be safely maintained for many division cycles without affecting cellular fitness.

One interesting and still unresolved issue is why cells polarize exactly within old division sites if inhibitory signals are removed (by deletion of Nba1 and Rga1) instead of simply overlapping with them, as may be expected with the relatively fast lateral diffusion of Cdc42 within the PM. A possible explanation comes from a recent study on spore germination in fission yeast (Bonazzi et al., 2014). In this system, a release in mechanical stress upon CW breakage reinforces stability of the polarized growth zone. Along a similar line, the rigid chitin-crosslinked rings around CRMs could reduce the stability of polarized caps and ensure accumulation of Cdc42 in the center of the ring.

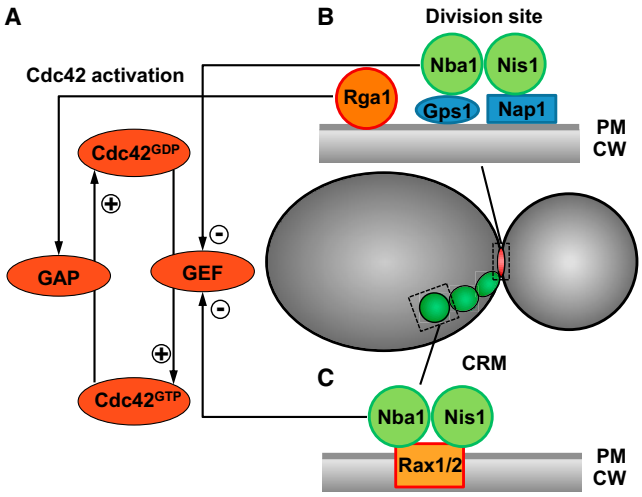

Figure 1. Mechanisms for Cdc42 Inactivation at Cortical Sites
Molecular interactions that mediate Cdc42 inactivation (A) at division sites and cytokinetic remnants (CRM) are shown schematically. Inhibition of Cdc42 at cell division sites (B) occurs through inhibition of the GEF Cdc24 by Nba1 and inactivation of Cdc42 by the GAP Rga1. In CRMs (C), Nba1 is the sole Cdc42 inhibitor. PM, plasma membrane; CW, cell wall; GAP, GTPase activating protein; GEF, GDP exchange factor.

A final question concerns the ultimate biological function of CRM formation. The elaborate inhibition of Cdc42 activity in CRMs and the severely reduced fitness upon removal of inhibition demonstrate the considerable costs and risks that a cell faces by establishing these structures. It is likely that dividing yeast cells require reinforcement of their secondary septa with chitin-based crosslinks to withstand the high osmotic pressure typical for fungi. Because polarity has to shift to a new growth site right after cytokinesis, perhaps the cell can then no longer secrete appropriate enzymes at the division site to degrade polymers within the bud scar. Subsequently, a cell may have to make use of the "necessary evil" of CRMs in functions unrelated to cytokinesis. In addition to their role in polarity determination, it has been suggested that bud scars contribute to cellular aging, although this effect has not yet been validated (Bitterman et al., 2003). In this context, the effect on replicative lifespan described by Meitinger et al. (2014) cannot be seen as a true aging phenotype, as cells without Cdc42 inhibition in CRMs simply end up dying as a result of geometric constriction at the bud neck, which becomes successively narrower with each renewed budding event. It therefore still remains to be seen whether CRMs truly have additional biological functions. Interestingly, analogous cytokinesis remnants such as the mitotic midbody have been identified in higher eukaryotes, and these have been implicated in processes such as cell polarization, intercellular communication, and cell differentiation (Chen et al., 2013).

In summary, the study by Meitinger et al. (2014) reveals a new facet of the ever-expanding repertoire of mechanisms that control cell polarity. It will now be an exciting task to determine the evolutionary diversity and biological implications of similar long-lasting memory cues throughout the various biological systems.

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